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I also certify that the attached copy of the request for grant of a Patent (Form 1) bears an amendment, effected by this office, following a request by the applicant and agreed to by the Comptroller-General.

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Dated 12 August 2008

Patents Form 1/77

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P01/7700 0.00-0121285.1

Request for grant of a patent

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The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference

P012392GB NJN

2. Patent application number
(The Patent Office will fill in this part)

0121285.1

03 SEP 2001

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

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Patents ADP number *(if you know it)*

78 22414002

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

ANTI-CANCER COMBINATIONS

5. Name of your agent *(if you have one)*

D Young & Co

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Patents ADP number *(if you know it)*

~~59006~~

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	1
Description	17
Claim(s)	3
Abstract	1
Drawing(s)	2 + 2 R N

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature D Young & Co. Date 03 September 200

D Young & Co (Agents for the Applicants)

12. Name and daytime telephone number of person to contact in the United Kingdom — Neil Nachshen — 020 7353 4343

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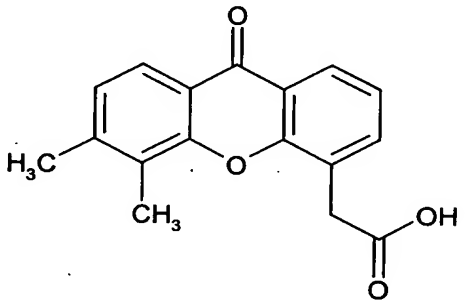
DUPLICATE

ANTI-CANCER COMBINATIONS

The present invention relates to synergistic combinations of the compound 5,6-dimethylxanthenone-4-acetic acid (DMXAA) and a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin, which have anti-tumour activity. More particularly, the invention is concerned with the use of such combinations in the treatment of cancer and pharmaceutical compositions containing such combinations.

5,6-dimethylxanthenone-4-acetic acid (DMXAA) is represented by the following formula:



Phase I clinical trials of DMXAA have recently been completed, with dynamic MRI showing that it induces a significant reduction in tumour blood flow at well-tolerated doses. DMXAA is thus one of the first antivascular agents for which activity (irreversible inhibition of tumour blood flow) has been documented in human tumours. These findings are in agreement with preclinical studies using tumours or human tumour xenografts which showed that its antivascular activity produced prolonged inhibition of tumour blood flow leading to extensive regions of haemorrhagic necrosis. However, in such studies tumours rapidly regrow from surviving cells in the well-perfused periphery. The transient tumour growth inhibition seen in most preclinical models is consistent with the lack of tumour regressions seen in the phase I clinical studies, and suggests that DMXAA is unlikely to have clinical utility as a single agent.

Carboplatin (Paraplatin®) is a platinum coordination cancer chemotherapeutic agent used primarily in the treatment of ovarian carcinoma. The chemical name for carboplatin is platinum, diammine [1,1-cyclobutanedicarboxylato(2-)-0, 0']-,(SP-4-2).

Gemcitabine (Gemzar®) (HCl) is a nucleoside analogue that exhibits antitumour activity. Gemcitabine HCl is 2'-deoxy-2',2'-difluorocytidine monohydrochloride (b-isomer).

Fluorouracil (Adrucil®) is an injectable antineoplastic antimetabolite. Its chemical name is 5-fluoro-2,4(1H,3H)-pyrimidinedione.

Cyclophosphamide (Cytoxan®) is available as a lyophilised cake for injection or as tablets for oral use. Cyclophosphamide is a synthetic antineoplastic drug chemically related to the nitrogen mustards. The chemical name for cyclophosphamide is 2-[bis(2-chloroethyl)amino]tetrahydro-2H-13,2-oxazaphosphorine-2-oxide monohydrate.

Doxorubicin HCl liposome injection (Doxil®) is a cytotoxic anthracycline antibiotic isolated from Streptomyces peucetius var. caesius. Doxil® (doxorubicin HCl liposome injection) is doxorubicin hydrochloride encapsulated in Stealth® liposomes for intravenous administration. It is indicated for the treatment of metastatic carcinoma of the ovary and the treatment of AIDS-related Kaposi's sarcoma and has the chemical name (8S,10S)-10-[(3-amino-2,3,6-trideoxy-a-L-lyxo-hexopyranosyl)oxy]-8-glycolyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride.

Cisplatin (Platinol®) is an antineoplastic agent used to treat a variety of tumour types.

Vincristine (Oncovin®, Vincasar PFS®, Vincrex®) is an antineoplastic given intravenously to treat Kaposi's sarcoma and non-Hodgkin's lymphoma.

Etoposide (VePesid®) (also commonly known as VP-16) is a semisynthetic derivative of podophyllotoxin used in the treatment of certain neoplastic diseases. It is 4'-demethylepipodophyllotoxin-9-[4,6-0-(R)-ethylidene-(beta)-D-glucopyranoside]. Etoposide is available for oral or intravenous administration and is indicated for refractory testicular tumours and small cell lung cancer.

It has now surprisingly been found that by combining, either concomitantly or sequentially, DMXAA with a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin potentiation of antitumour activity is achieved.

Thus, in a first aspect, the present invention provides a method for treating cancer, which comprises administering to a mammal, including a human, in need of such treatment an effective amount of DMXAA or a pharmaceutically acceptable salt or ester thereof and concomitantly or sequentially administering an effective amount of a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin.

In another aspect, the present invention provides the use of DMXAA or a pharmaceutically acceptable salt or ester thereof for the manufacture of a medicament, for administration either concomitantly or sequentially with a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin, for the treatment of cancer.

In a further aspect, the present invention provides the use of a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin for the manufacture of a medicament, for administration either concomitantly or sequentially with DMXAA or a pharmaceutically acceptable salt or ester thereof, for the treatment of cancer.

In a still further aspect, the present invention provides a combination of DMXAA or a pharmaceutically acceptable salt or ester thereof and a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin.

According to a still further aspect of the present invention, there is provided a kit comprising in association for separate administration DMXAA or a pharmaceutically acceptable salt or ester thereof and a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin.

Brief Description of the Drawings

Fig. 1. Illustrates growth delay of MDAH-Mca-4 tumours after treatment of mice with DMXAA alone (80 μmol/kg, i.p.), chemotherapeutic drug alone (i.p.), or co-administration of drug with DMXAA (80 μmol/kg). Values are mean ± sem for groups of 6-8 mice, ignoring deaths (d) or cures (c), the numbers of which are shown in parentheses.

Fig. 2. Left hand panel: Plasma concentrations of free platinum following administration of carboplatin alone (316 μmol/kg) (•), or co-administered with DMXAA (80 μmol/kg) (O). Right hand panel: Tumour concentrations of total platinum following administration of carboplatin alone (316 μmol/kg) (•), or co-administered with DMXAA (80 μmol/kg) (O).

The DMXAA or pharmaceutically acceptable salt or ester thereof and the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin may be administered concomitantly or sequentially. Preferably the DMXAA or pharmaceutically acceptable salt or ester thereof and the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin are administered concomitantly.

Preferably the DMXAA or pharmaceutically acceptable salt or ester thereof and the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin are present in a potentiating ratio.

The term 'potentiating ratio' is used herein to indicate that the DMXAA or pharmaceutically acceptable salt or ester thereof and the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin are present in a ratio such that the antitumour activity of the combination is greater than that of DMXAA alone or the compound selected form carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin alone or of the additive activity that would be predicted for the combinations based on the activities of the individual components. Thus the individual components act synergistically in combination provided they are present in a potentiating ratio.

A potentiating ratio, for DMXAA and fluorouracil which may be successfully used to treat cancer, is in the range 1:100 to 1:2 of DMXAA:fluorouracil. Suitably, the potentiating ratio is in the range 1:75 to 1:5. A further potentiating ratio is in the range 1:50 to 1:10. A preferred potentiating ratio is in the range 1:30 to 1:15, more preferably in the range 1:25 to 1:20 of DMXAA:fluorouracil.

A potentiating ratio, for DMXAA and carboplatin which may be successfully used to treat cancer, is in the range 1:20 to 1:1 of DMXAA:carboplatin. Suitably, the potentiating ratio is in the range 1:16 to 1:2. A further potentiating ratio is in the range 1:10 to 1:2. A preferred potentiating ratio is in the range 1:8 to 1:3, more preferably in the range 1:6 to 1:4 of DMXAA:carboplatin.

A potentiating ratio, for DMXAA and cisplatin which may be successfully used to treat cancer, is in the range 20:1 to 1:1 of DMXAA:cisplatin. Suitably, the potentiating ratio

is in the range 10:1 to 1:1. A further potentiating ratio is in the range 8:1 to 1:1. A preferred potentiating ratio is in the range 6:1 to 2:1, more preferably in the range 4:1 to 2:1 of DMXAA:cisplatin.

A potentiating ratio, for DMXAA and cyclophosphamide which may be successfully used to treat cancer, is in the range 1:100 to 1:2 of DMXAA:cyclophosphamide. Suitably, the potentiating ratio is in the range 1:50 to 1:5. A further potentiating ratio is in the range 1:30 to 1:5. A preferred potentiating ratio is in the range 1:20 to 1:8, more preferably in the range 1:16 to 1:12 of DMXAA:cyclophosphamide.

A potentiating ratio, for DMXAA and etoposide which may be successfully used to treat cancer, is in the range 10:1 to 1:10 of DMXAA:etoposide. Suitably, the potentiating ratio is in the range 5:1 to 1:5. A further potentiating ratio is in the range 5:1 to 1:3. A preferred potentiating ratio is in the range 3:1 to 1:2, more preferably in the range 2:1 to 1:2 of DMXAA:etoposide.

A potentiating ratio, for DMXAA and vincristine which may be successfully used to treat cancer, is in the range 200:1 to 5:1 of DMXAA:vincristine. Suitably, the potentiating ratio is in the range 150:1 to 10:1. A further potentiating ratio is in the range 100:1 to 40:1. A preferred potentiating ratio is in the range 100:1 to 60:1, more preferably in the range 90:1 to 70:1 of DMXAA:vincristine.

A potentiating ratio, for DMXAA and doxorubicin which may be successfully used to treat cancer, is in the range 50:1 to 1:1 of DMXAA:doxorubicin. Suitably, the potentiating ratio is in the range 25:1 to 1:1. A further potentiating ratio is in the range 16:1 to 2:1. A preferred potentiating ratio is in the range 8:1 to 2:1, more preferably in the range 6:1 to 4:1 of DMXAA:doxorubicin.

Preferably the pharmaceutically acceptable salt is the sodium salt.

In one embodiment the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin is carboplatin.

In one embodiment the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin is gemcitabine.

In one embodiment the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin is cisplatin.

In one embodiment the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin is fluorouracil.

In one embodiment the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin is cyclophosphamide.

In one embodiment the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin is etoposide.

In one embodiment the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin is vincristine.

In one embodiment the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin is doxorubicin.

The amount of a combination of DMXAA or a pharmaceutically acceptable salt or ester thereof and a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin required to be effective as an anticancer agent will, of course, vary and is ultimately at the discretion of the medical practitioner. The factors to be considered include the route of administration and nature of the formulation, the mammal's bodyweight, age and general condition and the nature and severity of the disease to be treated.

In general, a suitable effective dose of DMXAA, or a pharmaceutically acceptable salt thereof, for administration, either concomitantly or sequentially, with a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin to man for the treatment of cancer is in the range of 600 to 4900 mg/m^2. For example from 2500 to 4000 mg/m^2, suitably from 1200 to 3500 mg/m^2, more suitably from 2000 to 3000 mg/m^2, particularly from 1200 to 2500 mg/m^2, more particularly from 2500 to 3500 mg/m^2, preferably from 2250 to 2750 mg/m^2. Preferably the DMXAA, or pharmaceutically acceptable salt thereof, is administered by IV once every week or every 3 weeks.

In general, a suitable effective dose of carboplatin for administration, either concomitantly or sequentially, with DMXAA, or a pharmaceutically acceptable salt thereof, to man for the treatment of cancer is in the range 100 to 500 mg/m^2. For example

from 100 to 300 mg/m^2, suitably from 250 to 400 mg/m^2, more suitably from 150 to 350 mg/m^2, particularly from 150 to 250 mg/m^2, more particularly from 175 to 225 mg/m^2. Preferably carboplatin is administered by IV once every 4 weeks.

In general, a suitable effective dose of gemcitabine for administration, either concomitantly or sequentially, with DMXAA, or a pharmaceutically acceptable salt thereof, to man for the treatment of cancer is in the range 400 to 2000 mg/m^2. For example from 500 to 1500 mg/m^2, suitably from 600 to 1200 mg/m^2, more suitably from 600 to 1000 mg/m^2, particularly from 800 to 1200 mg/m^2, more particularly from 800 to 1000 mg/m^2, preferably from 750 to 950 mg/m^2. Preferably gemcitabine is administered by IV once every week.

In general, a suitable effective dose of cisplatin for administration, either concomitantly or sequentially, with DMXAA, or a pharmaceutically acceptable salt thereof, to man for the treatment of cancer is in the range 10 to 200 mg/m^2. For example from 20 to 150 mg/m^2, suitably from 30 to 120 mg/m^2, more suitably from 40 to 100 mg/m^2, particularly from 40 to 80 mg/m^2, more particularly from 60 to 100 mg/m^2, preferably from 75 to 100 mg/m^2. Preferably cisplatin is administered by IV once every 4 weeks.

In general, a suitable effective dose of fluorouracil for administration, either concomitantly or sequentially, with DMXAA, or a pharmaceutically acceptable salt thereof, to man for the treatment of cancer is in the range 2 to 20 mg/kg. For example from 2 to 15 mg/kg, suitably from 2 to 8 mg/kg, more suitably from 6 to 12 mg/kg, particularly from 4 to 10 mg/kg, preferably from 4 to 6 mg/kg. Preferably fluorouracil is administered on alternate days for a period of about 2 weeks.

In general, a suitable effective dose of cyclophosphamide for administration, either concomitantly or sequentially, with DMXAA, or a pharmaceutically acceptable salt thereof, to man for the treatment of cancer is in the range 100 to 1000 mg/m^2. For example from 200 to 800 mg/m^2, suitably from 200 to 500 mg/m^2, more suitably from 350 to 700 mg/m^2, particularly from 450 to 650 mg/m^2, more particularly from 500 to 600 mg/m^2, preferably from 550 to 650 mg/m^2. Preferably cyclophosphamide is administered by IV once every 4 weeks.

In general, a suitable effective dose of etoposide for administration, either concomitantly or sequentially, with DMXAA, or a pharmaceutically acceptable salt thereof, to man for the treatment of cancer is in the range 5 to 150 mg/m^2. For example from 5 to 120 mg/m^2, suitably from 10 to 100 mg/m^2, more suitably from 15 to 50 mg/m^2, particularly

from 60 to 120 mg/m^2, more particularly from 35 to 75 mg/m^2, preferably from 30 to 60 mg/m^2. Preferably etoposide is administered by IV daily for 4 to 7 days.

In general, a suitable effective dose of vincristine for administration, either concomitantly or sequentially, with DMXAA, or a pharmaceutically acceptable salt thereof, to man for the treatment of cancer is in the range 0.1 to 2.0 mg/m^2. For example from 0.125 to 1.75 mg/m^2, suitably from 0.15 to 1.5 mg/m^2, more suitably from 0.2 to 1.4 mg/m^2, particularly from 0.6 to 1.4 mg/m^2, more particularly from 0.8 to 1.4 mg/m^2, preferably from 0.5 to 1.0 mg/m^2. Preferably vincristine is administered by IV once every week.

In general, a suitable effective dose of doxorubicin for administration, either concomitantly or sequentially, with DMXAA, or a pharmaceutically acceptable salt thereof, to man for the treatment of cancer is in the range 5 to 100 mg/m^2. For example from 10 to 80 mg/m^2, suitably from 20 to 60 mg/m^2, more suitably from 40 to 75 mg/m^2, particularly from 20 to 50 mg/m^2, more particularly from 15 to 35 mg/m^2, preferably from 40 to 60 mg/m^2. Preferably doxorubicin is administered by IV once every 3-4 weeks.

The DMXAA or pharmaceutically acceptable salt or ester thereof and the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin may be administered in any suitable form. However, for use according to the present invention the combination of DMXAA or a pharmaceutically acceptable salt or ester thereof and a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin is preferably presented as a pharmaceutical formulation.

Pharmaceutical formulations comprise the active ingredients (that is, the combination of DMXAA or a pharmaceutically acceptable salt or ester thereof and a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin) together with one or more pharmaceutically acceptable carriers therefor and optionally other therapeutic and/or prophylactic ingredients. The carrier(s) must be acceptable in the sense of being compatible with the other ingredients of the formula and not deleterious to the recipient thereof.

Accordingly, the present invention provides a pharmaceutical formulation comprising a combination of DMXAA or a pharmaceutically acceptable salt or ester thereof and a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin in association with one or more pharmaceutically acceptable carriers therefor.

The present invention further provides a process for the preparation of a pharmaceutical formulation which process comprises bringing into association a combination of DMXAA or a pharmaceutically acceptable salt or ester thereof and a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin with one or more pharmaceutically acceptable carriers therefor.

Pharmaceutical formulations include those suitable for oral, topical (including dermal, buccal and sublingual), rectal and parenteral (including subcutaneous, intradermal, intramuscular and intravenous) administration as well as administration by naso-gastric tube. The formulation may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

Preferably the pharmaceutical formulations is adapted for parenteral administration, most preferably intravenous administration. For example the compounds may be administered intravenously using formulations for each compound already known in the art.

Pharmaceutical formulations suitable for oral administration wherein the carrier is a solid are most preferably presented as unit dose formulations such as boluses, capsules or tablets each containing a predetermined amount of the active ingredients. A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compounds in a free-flowing form such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, lubricating agent, surface-active agent or dispersing agent. Moulded tablets may be made by moulding an inert liquid diluent. Tablets may be optionally coated and, if uncoated, may optionally be scored. Capsules may be prepared by filling the active ingredients, either alone or in admixture with one or more accessory ingredients, into the capsule shells and then sealing them in the usual manner. Cachets are analogous to capsules wherein the active ingredients together with any accessory ingredient(s) are sealed in a rice paper envelope. The combination of DMXAA or a pharmaceutically acceptable salt or ester thereof and a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin may also be formulated as dispersible granules, which may for example be suspended in water before administration, or sprinkled on food. The

granules may be packaged e.g. in a sachet. Formulations suitable for oral administration wherein the carrier is a liquid may be presented as a solution or a suspension in an aqueous liquid or a non-aqueous liquid, or as an oil-in-water liquid emulsion.

Formulations for oral administration include controlled release dosage forms e.g. tablets wherein the active ingredients are formulated in an appropriate release - controlling matrix, or are coated with a suitable release - controlling film. Such formulations may be particularly convenient for prophylactic use.

The active ingredients may also be formulated as a solution or suspension suitable for administration via a naso-gastric tube.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art. The suppositories may be conveniently formed by admixture of the active combination with the softened or melted carrier(s) followed by chilling and shaping in moulds.

Pharmaceutical formulations suitable for parenteral administration include sterile solutions or suspensions of the active combination in aqueous or oleaginous vehicles. Injectible preparations may be adapted for bolus injection or continuous infusion. Such preparations are conveniently presented in unit dose or multi-dose containers which are sealed after introduction of the formulation until required for use. Alternatively, the active ingredients may be in powder form which are constituted with a suitable vehicle, such as sterile, pyrogen-free water, before use.

The combination of DMXAA or a pharmaceutically acceptable salt or ester thereof and compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin may also be formulated as a long-acting depot preparation, which may be administered by intramuscular injection or by implantation e.g. subcutaneously or intramuscularly. Depot preparations may include, for example, suitable polymeric or hydrophobic materials, or ion-exchange resins. Such long-acting formulations are particularly convenient for prophylactic use.

It should be understood that in addition to the aforementioned carrier ingredients the pharmaceutical formulations for the various routes of administration described above may include, as appropriate one or more additional carrier ingredients such as diluents, buffers, flavouring agents, binders, surface active agents, thickeners, lubricants,

preservatives (including anti-oxidants) and the like, and substances included for the purpose of rendering the formulation isotonic with the blood of the intended recipient.

DMXAA may be prepared according to the methods described in Journal of Medicinal Chemistry 34(1): 217-22, January 1991 the contents of which are incorporated herein by reference.

Carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin are well known compounds and may be prepared by methods known to those skilled in the art.

It is to be understood that the present invention covers all combinations of suitable and preferred groups described hereinabove.

The present invention will now be illustrated, but is not intended to be limited, by means of the following examples.

EXAMPLES

Materials And Methods

Compounds: A stock solution of DMXAA, synthesised in the Auckland Cancer Society Research Centre, was prepared in phosphate-buffered saline, protected from light and stored frozen. Cisplatin (Sigma Chemical Co., St Louis, MO) was dissolved in 0.9% saline. Stock solutions of carboplatin and fluorouracil (Bristol Myers Squibb, Sermonita, Italy) and cyclophosphamide (Mead Johnson Oncology Products, Princeton, NJ) were diluted with sterile water. Doxorubicin (Farmitalia Carlo Erba Pty Ltd, Clayton North, Australia), etoposide, and vincristine (Bristol-Myers Squibb, Sermonita, Italy) were diluted using 0.9% saline. All compounds were administered to mice by i.p. injection at 0.01 ml/g body weight.

Animals and Tumours: Murine mammary carcinoma MDAH-Mca-4 tumours were grown from stocks stored in liquid nitrogen at the sixth transplant generation. Tumours (eighth transplant generation when used) were grown from 20 µl of cell suspension (7 mg packed cells), inoculated i.m. in the right gastrocnemius muscle of female C_3H/HeN mice (22-25 g at the time of treatment). Mice were randomised to treatment which commenced when the tumour + leg diameter reached 10-11 mm (0.5-0.7 g tumour).

Host Toxicity and Antitumour Activity: Mice were treated with chemotherapeutic drugs at a range of doses, at 1.33-fold increments, up to the expected MTD (maximum tolerated dose, as estimated in pilot experiments or from the literature). Toxicity was assessed as lethality, and body weight loss measured four days after treatment. Any animals becoming moribund were terminated and treated as drug-related deaths in the analysis. The diameter of the tumour-bearing leg was measured 3 times per week after treatment. Antitumour activity was assessed from the tumour growth delay, defined as the difference in time to reach the endpoint of 13 mm (1.5 g tumour) for treated and control groups. Responses were classed as cures if animals were free of evident tumour 120 days after treatment. The statistical significance of tumour growth inhibition was tested by ANOVA using SAS for Windows, with Dunnett's test to evaluate *p*-values for differences between treatment groups. In experiments with substantial numbers of cures (free of tumour for > 120 days after treatment), statistical significance was tested by Kruskal-Wallis non-parametric analysis of variance using SAS for Windows, and the difference between treatment and control groups by Dunn's test using Sigmastat v2.0. The gradient and standard error of dose-response curves was determined by linear regression using Sigmastat, and the DMF calculated as the gradient with DMXAA/gradient without DMXAA.

Tumour blood flow inhibition: Tumour blood flow was assessed using the fluorescent perfusion marker Hoechst 33342 (8mg/ml in saline), which was administered i.v. at various times after drug treatment. Mice were scarified 2 min later, and frozen sections (14 μm) prepared from the distal, central and proximal regions of each tumour. Sections were examined with a Nikon epifluorescence microscope at 10x magnification using a UV-1A filter block (excitation 365 nm, barrier filter 400 nm, and dichroic mirror 400 nm). A grid with 81 squares (100 x 100 μm), was used for point scoring of staining. The whole area of each section was scored to avoid bias between peripheral and central regions (which were less well perfused). Normal tissue was excluded but necrotic areas were included. Differences between groups were treated for significance using the Student's *t*-test (Sigma Stat, version-2.0; Jandel Scientific Limited).

Pharmacokinetics: Female C_3H/HeN mice bearing MDAH-Mca-4 tumours (0.5-0.7 g) were injected i.p. with carboplatin (316 μmol/kg), DMXAA (80 μmol/kg), or simultaneously received carboplatin and DMXAA, at the same doses. At various times blood was collected from the retro-orbital sinus of anaesthetised mice into heparinised tubes, and the plasma separated by centrifugation. Tumours were rapidly dissected and frozen at -80°C. Groups of 2-5 mice were used for each time point.

ICP-MS analysis of platinum: Concentrations of platinum in plasma and tumours were determined using the following previously published ICP-MS method. Tumours were weighted, placed in 15 ml screw cap tubes containing 1ml of 70% nitric acid (Riedel-de-Haen, Seelze, Germany), and left to stand overnight at room temperature. The following day, tumours were digested for 2 h at 90°C in a sand-filled electric frying pan positioned with a fume hood. After cooling, the solubilized tumour tissues were made to volume in 10-ml volumetric flasks using Milli-Q water and then introduced into the ICP-MS. Plasma was prepared for analysis by methanol precipitation of plasma protein. Plasma was added to an equal volume of ice-cold methanol, mixed and left to stand at -20°C for 18 h. The sample was centrifuged and an aliquot of supernatant was diluted (1:40) in 0.1% nitric acid before being introduced into the ICP-MS.

The ICP-MS system comprised a Hewlett Packard HP 4500 ICP-MS with a nickel sampling cone, Babington (v-groove) nebulizer and a Scott double-pass spray chamber maintained at 2°C. Platinum was read at 195 amu with a dwell time of 100 ms and a replicate time of 6000 ms. Calibration curves were linear (r^2>0.98) over a wide range (0.5 to 5000 ng/ml). Intra-assay and inter-assay variability and recovery were within acceptable limits. The limits of quantitation were 12.5 pg of platinum per ml of plasma and 10 ng of platinum per g of tumour tissue.

HPLC analysis of DMXAA: Concentrations of DMXAA in plasma were determined as follows. Aliquots of plasma (50 µl) were treated with 1 ml of ice-cold acetonitrile:methanol (3:1 v/v), centrifuged, and the resulting supernatants evaporated as above. The residues were dissolved in 200 µl of 10 mM ammonium acetate buffer (pH 5) and 25 µl was analysed by HPLC using a HP1100 system with diode array detector (278 nm) and fluorescence detector. The column used was a 3.2 mm x 150 mm C_8 5 µm column (Alltima Associates Inc., Deerfield, IL) and a flow rate of 0.7 ml/min, with a mobile phase of 16% acetonitrile (v/v) in 10 mM ammonium acetate buffer (pH 5). The retention time for DMXAA was 7.3 min. Spiking of control plasma showed assay linearity from 0.1-100 µM (r^2 = 0.999). The intra- and inter- assay precision and accuracy gave coefficients of variation <7%, and an average recovery of 70%. The lower sensitivity limit of detection by fluorescence (signal:noise ration of 3) was 0.1 µM.

Pharmacokinetic Modelling: Modelling of pharmacokinetic data was done using ModelMaker version 4.0 (Cherwell Scientific Limited, The Magdalen Centre, Oxford Science Park, Oxford OX4 4GA, United Kingdom). The following pharmacokinetic parameters were used: K_{abs}, the first-order rate constant for absorption into the central compartment; Cl, total body clearance; Cl_{inter}, intercompartmental clearance; V_d,

apparent volume of distribution of the central compartment; V_{d2}, apparent volume of distribution of the second compartment; K_m, Michaelis-Menten constant; V_{max}, theoretical maximum rate; AUC, area under the concentration-time curve. For all compounds it was assumed that all of the administered dose would reach the central compartment (i.e. 100% bioavailability). Differences between treatment groups were tested using a F-test comparing the entire curves and if this difference was significant (i.e. $p \leq 0.05$) the estimates of each individual model parameter for both groups were tested using a 2-tailed t-test. The concentrations of free platinum and total platinum in plasma and tumour were fiitted with a 1-compartment open model assuming linear pharmacokinetics. Plasma concentrations of DMXAA were fitted using a 1-compartment open model with saturable (Michaelis-Menten) elimination kinetics.

Results

Activity of DMXAA + chemotherapy drugs against MDAH-Mca-4 tumour. The antitumour activity and host toxicity of DMXAA/cytotoxic drug combinations was assessed by varying the dose of chemotherapeutic drug up to the toxicity limit, with co-administration of a fixed DMXAA dose (80μmol/kg, ca. 80% of MTD), and evaluating subsequent tumour growth delay, as illustrated in Fig 1. Of the seven drugs investigated, four (doxorubicin, 5-fluorouracil, cyclophosphamide and cisplatin) showed appreciable activity against this tumour as indicated by dose-response relationships providing significant slopes by linear regression, and highly significant growth delays of ca 10 days at their MTDs (which are recorded in Table 1). The other three compounds (carboplatin, etoposide and vincristine) were essentially inactive, with no individual treatment groups showing significant activity (although carboplatin gave weakly positive dose responses by linear regression).

DMXAA alone showed appreciable activity as a single agent at 80 μmol/kg, providing transient regressions and mean tumour growth delays in the range 3.5 – 8.3 days (overall mean 6.6 ± 0.6 days). Co-administration of DMXAA at this dose increased the host toxicity of doxorubicin and the MTD for the chemotherapy drug was lowered by one dose level (1:33-fold) in the combination (Table 1). For the other compounds, co-administration of DMXAA did not alter the formal MTD although some additional toxicity was evident as indicated by the greater body weight loss in the combination.

In contrast to this small effect on host toxicity, co-administration of DMXAA produced a large enhancement of tumour growth delay (Table 1). The contribution of DMXAA was assessed by determining the slope of each dose-response curve by linear regression, and

the DMF for DMXAA was calculated as the ratio of slopes with and without DMXAA. By this criterion the magnitude of the synergy decreased in the order vincristine > (carboplatin, cisplatin, cyclophosphamide, etoposide, doxorubicin) > fluorouracil. For each of these compounds, except the later, the DMF was significantly greater then unity. As an alternative criterion, the maximum tumour growth delay achievable at the MTD of the combination again indicated synergy for all the compounds with growth delays in the range 15-30 days.

Pharmacokinetics of DMXAA and carboplatin: Studies were conducted to deduce whether a pharmacokinetic interaction underlies the synergistic therapeutic interaction between DMXAA and carboplatin. The study was conducted in C_3H mice bearing MDAH-Mca-4 tumours of the same size as in the therapeutic studies. Following administration of carboplatin 316 μmol/Kg, i.p.) clearance of Pt from plasma (measured, after deproteinization, by ICP-MS) was biphasic, and was unaffected by co-administration of DMXAA (Fig 2). Total Pt in the tumour also showed biphasic kinetics which were unaffected by co-administration of DMXAA. The lack of effect of DMXAA was confirmed by modelling the plasma with tumour pharmacokinetics as a 2-compartment open model with linear pharmacokinetics, which provided the model parameters of Table 2 and indicated that there is no significant effect of DMXAA.

This study tests the hypothesis that antivascular agents such as DMXAA have the potential to combine synergistically with conventional cytotoxic agents in the treatment of solid tumours. The early passage mammary tumour MDAH-Mca-4, used for this comparative study, was moderately refractory to most of the cytotoxic drugs tested (using single drug doses) but showed significant responses to doxorubicin, 5-fluorouracil, cyclophosphamide and cisplatin. DMXAA alone showed consistent activity as a single agent, of similar magnitude to these four agents, but neither the chemotherapy drugs nor DMXAA provided prolonged regressions or cures.

However, co-administration of DMXAA with the cytotoxic drugs caused a marked increase in response (Fig. 1). This interaction can be classified as synergistic (super-additive) on the basis of the increased slope of the cytotoxic drug dose response curve on addition of DMXAA. The interaction, quantified as the DMF (ratio of the linear regression slopes with and without DMXAA), was significantly greater than unity for all drugs except 5-fluorouracil. It is noteworthy that the interaction with DMXAA resulted in substantial activity with several compounds which did not show any single agent activity.

Table 1 Effect of DMXAA on host toxicity and antitumour activity of chemotherapeutic drugs against MDAH-Mca-4 tumours. Drugs were co-administered with DMXAA by i.p. injection

Chemotherapy drug	DMXAA (μmol/kg)	MTD (μmol/kg)	% body weight change at 4 days	Slope of dose/response (days/ μmol/kg)	DMF
Fluorouracil	-	1780	-8.5±0.8	0.0051±0.0001[a]	0.5±0.2
	80	1780	-14.1±1.5	0.0027±0.001	
Carboplatin	-	316	-5.6±1.5	0.0094±0.0035	3.4±2.3
	80	316	-8.5±1.4	0.032±0.010	
Cisplatin	-	42.1	-9.5±1.4	0.19±0.06	1.8±1.2
	80	42.1	-14.4±2.3	0.35±0.12	
Cyclo-phosphamide	-	≥1335	-0.8±1.3	0.0062±0.0001	2.7±0.3
	80	≥1335	-9.2±1.4	0.0167±0.0013	
Etoposide	-	≥75	-2.0±1.7	0.030±0.010	4.7±2.9
	80	75(1d)	-6.5±3.3	0.14±0.04	
Vincristine	-	1.0	-7.0±1.2	-0.0±1.3	>7[c]
	80	1.0	-10.0±1.4	14.1±5.4	
Doxorubicin	-	23.7	-3.9±0.6	0.42±0.10	2.5±1.1
	80	17.8	-5.5±1.2	1.04±0.23	

[a] Standard error of the slope
[c] Estimated using upper error estimate of the slope for the chemotherapy drug only.

Table 2. Pharmacokinetic parameters for Carboplatin (316 μmol/kg), and DMXAA (80 μmol/kg) in plasma and tumour of female C_3H/HeN mice bearing MDAH-Mca-4 tumours (ca 0.7g). Numbers in parentheses are % CV.

Parameter	Plasma		Tumour
	Carboplatin	DMXAA	Carboplatin
K_{abs} (hr^{-1})	12.6 (266)	9.3 (39)	14.0 (286)
K_m	-	220 (9.2)	-
V_{max} ($\mu M\ hr^{-1}$)	-	63 (6.1)	-
Cl ($1\ hr^{-1}\ kg^{-1}$)	1.9 (41)	-	0.28 (31)
Cl_{inter} ($1\ hr^{-1}\ kg^{-1}$)	0.41(99)	-	2.3 (24)
V_d $1\ kg^{-1}$	1.0 (49)	0.17 (1.2)	2.7 (19)
V_d ($1\ kg^{-1}$) (coadmin.)	-	-	-
V_{d2} ($1\ kg^{-1}$)	7.4(227)	-	11.3 (1163)
AUC ($\mu M.hr$)[a]	112[b]	3628[c]	416[b]
AUC ($\mu M.hr$)[a] (coadmin.)	118[b,c]	3136[c]	475[b,c]

[a] AUC was calculated using the linear trapezoidal rule
[b] 0-24 hours
[c] Coadministration with DMXAA
[d] 0-8 hours
[e] 0-30 hours

Claims

1. A method for treating cancer, which comprises administering to a mammal, including a human, in need of such treatment an effective amount of DMXAA or a pharmaceutically acceptable salt or ester thereof and concomitantly or sequentially administering an effective amount of a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin.

2. A method according to claim 1 wherein the DMXAA or pharmaceutically acceptable salt or ester thereof and the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin are administered in a potentiating ratio.

3. A method according claim 1 or claim 2 wherein the DMXAA or pharmaceutically acceptable salt or ester thereof and the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin are administered concomitantly.

4. A method according claim 1 or claim 2 wherein the DMXAA or pharmaceutically acceptable salt or ester thereof and the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin are administered sequentially.

5. Use of DMXAA or a pharmaceutically acceptable salt or ester thereof for the manufacture of a medicament, for administration either concomitantly or sequentially with a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin, for the treatment of cancer.

6. Use of a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin for the manufacture of a medicament, for administration either concomitantly or sequentially with DMXAA or a pharmaceutically acceptable salt or ester thereof, for the treatment of cancer.

7. Use according to claim 5 or claim 6 wherein the DMXAA or pharmaceutically acceptable salt or ester thereof and the compound selected from carboplatin,

gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin are present in a potentiating ratio.

8. Use according to any of claims 5 to 7 wherein the DMXAA or pharmaceutically acceptable salt or ester thereof and the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin are administered concomitantly.

9. Use according to any of claims 5 to 7 wherein the DMXAA or pharmaceutically acceptable salt or ester thereof and the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin are administered sequentially.

10. A combination of DMXAA or a pharmaceutically acceptable salt or ester thereof and a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin.

11. A combination according to claim 10 wherein the of DMXAA or a pharmaceutically acceptable salt or ester thereof and the compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin are present in a potentiating ratio.

12. A pharmaceutical formulation comprising a combination of DMXAA or a pharmaceutically acceptable salt or ester thereof and a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin in association with one or more pharmaceutically acceptable carriers therefor.

13. A pharmaceutical formulation according to claim 12 wherein the formulation is adapted for intravenous administration.

14. A process for the preparation of a pharmaceutical formulation which process comprises bringing into association a combination of DMXAA or a pharmaceutically acceptable salt or ester thereof and a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin with one or more pharmaceutically acceptable carriers therefor.

15. A kit comprising in association for separate administration DMXAA or a pharmaceutically acceptable salt or ester thereof and a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin.

ABSTRACT

ANTI-CANCER COMBINATIONS

The present invention relates to synergistic combinations of the compound 5,6-dimethylxanthenone-4-acetic acid (DMXAA) and a compound selected from carboplatin, gemcitabine, cisplatin, fluorouracil, cyclophosphamide, etoposide, vincristine and doxorubicin, which have anti-tumour activity. More particularly, the invention is concerned with the use of such combinations in the treatment of cancer and pharmaceutical compositions containing said combinations.

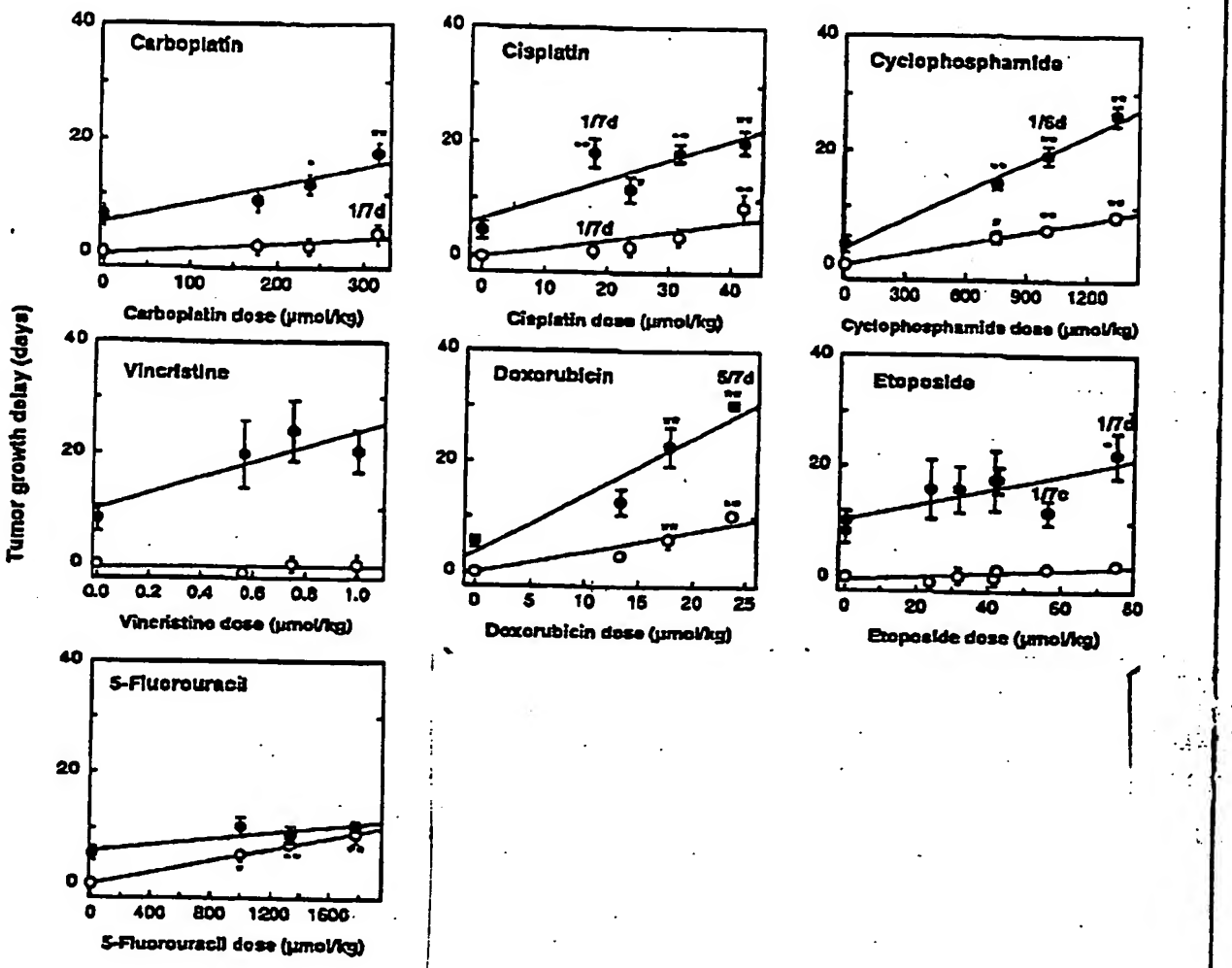
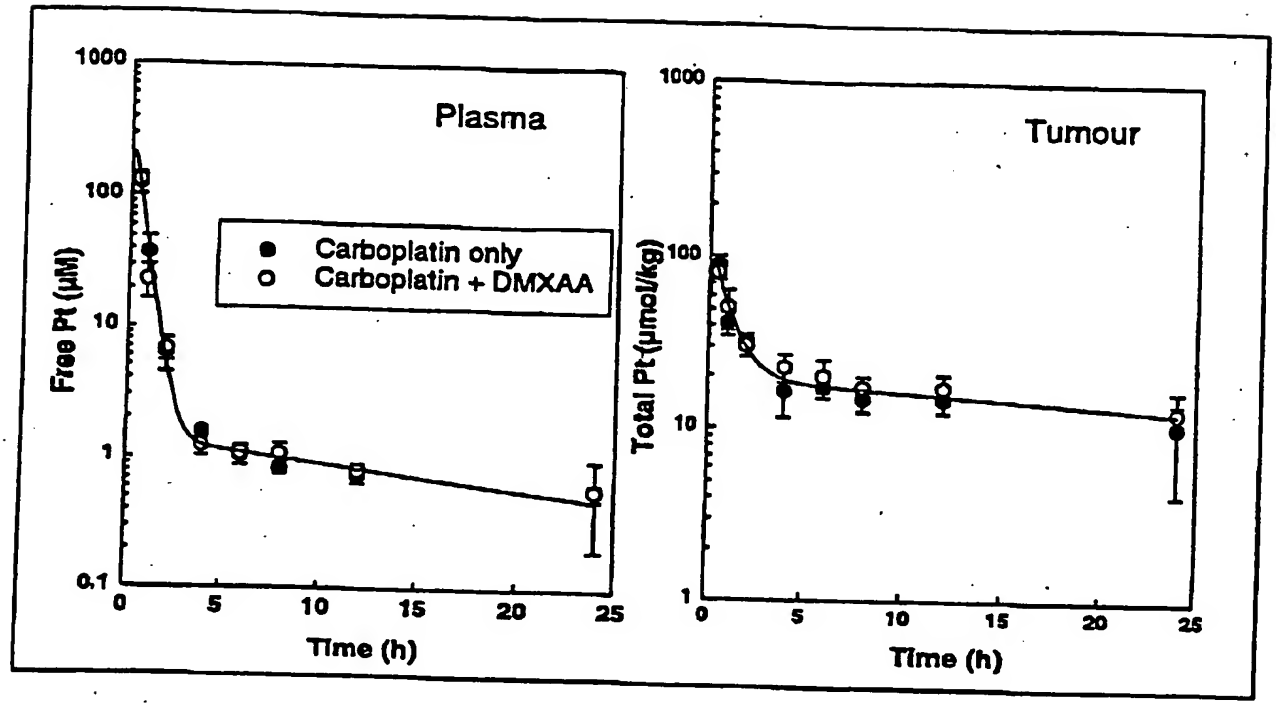

Carboplatin
1/7d
Carboplatin dose (μmol/kg)
Cisplatin
1/7d
1/7d
Cisplatin dose (μmol/kg)
Cyclophosphamide
1/6d
Cyclophosphamide dose (μmol/kg)
Vincristine
Vincristine dose (μmol/kg)
Doxorubicin
5/7d
Doxorubicin dose (μmol/kg)
Etoposide
1/7d
1/7c
Etoposide dose (μmol/kg)
5-Fluorouracil
5-Fluorouracil dose (μmol/kg)
Tumor growth delay (days)

Fig 1

Fig 2


Plasma
Carboplatin only
Carboplatin + DMXAA
Free Pt (μM)
Time (h)
Tumour
Total Pt (μmol/kg)
Time (h)